☐1: L27092. Pneumocystis cari...[gi:535706] Related Sequences, Protein, PubMed, Taxonomy

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REFERENCE   1  (bases 1 to 3363)
  AUTHORS   Garbe,T.R. and Stringer,J.R.
  TITLE     Molecular characterization of clustered variants of genes encoding
            major surface antigens of human Pneumocystis carinii
  JOURNAL   Infect. Immun. 62 (8), 3092-3101 (1994)
  MEDLINE   94314421
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Revised: October 24, 2001.

☐1: U34351. Pneumocystis cari...[gi:1144496] Protein, PubMed, Taxonomy

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SOURCE      Pneumocystis carinii.
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REFERENCE   1  (bases 1 to 306)
  AUTHORS   Wright,T.W., Gigliotti,F., Haidaris,C.G. and Simpson-Haidaris,P.J.
  TITLE     Cloning and characterization of a conserved region of human and
            rhesus macaque Pneumocystis carinii gpA
  JOURNAL   Gene 167 (1-2), 185-189 (1995)
  MEDLINE   96144272
   PUBMED   8566775
REFERENCE   2  (bases 1 to 306)
  AUTHORS   Wright,T.W.
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  JOURNAL   Submitted (21-AUG-1995) Terry W. Wright, Microbiology and
            Immunology, University of Rochester School of Medicine and
            Dentistry, 601 Elmwood Avenue, Rochester, NY 14642, USA
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Mol Cell Probes 1991 Apr;**5(2)**:143-149

The use of europium (Eu3+) labelled primers in PCR amplification of specific target DNA.

Dahlen P, Iitia A, Mukkala VM, Hurskainen P, Kwiatkowski M

Pharmacia Genetic Engineering Inc., La Jolla, CA 92037.

The polymerase chain reaction (PCR) has many potential applications in the field of DNA probe diagnostics. Here we describe a method that utilizes PCR and time-resolved fluorometry (TRF) for the detection of specific target DNA. First the DNA segment to be detected is amplified according to standard procedures. Then a pair of europium (Eu3+) and biotin-labelled primers nested within the amplified fragment is incorporated in a few additional PCR cycles. Thus amplified DNA fragments are generated that contain an affinity label (biotin) and a detectable label (europium). The doubly-labelled amplified DNA fragments are collected onto streptavidin coated microtitration strips and the bound Eu3+ is measured in a time-resolved fluorometer. We show here the application of this method to the detection of HIV-1 DNA. As few as five copies of HIV-1 DNA could readily be detected using this assay. The method described here is sensitive, rapid and easy to employ. In addition it lends itself to automation.

PMID: 2072935, UI: 91304446

J Clin Microbiol 1991 Apr;**29(4)**:798-804

Detection of human immunodeficiency virus type 1 by using the polymerase chain reaction and a time-resolved fluorescence-based hybridization assay.

Dahlen PO, Iitia AJ, Skagius G, Frostell A, Nunn MF, Kwiatkowski M

Pharmacia Genetic Engineering Inc., La Jolla, California 92037.

The polymerase chain reaction (PCR) has many potential applications in the field of nucleic acid diagnostics. In particular, it has been successfully applied to the detection of pathogens present in low copy numbers such as the human immunodeficiency virus type 1. Here we describe a time-resolved fluorescence-based hybridization assay which, combined with the PCR, offers an extremely sensitive method for the detection of nucleic acids. In this assay format, the PCR is run by standard procedures and the subsequent hybridization reaction is carried out in solution by using two oligonucleotide probes, one biotinylated and one labeled with europium (Eu3+). The sandwich hybrids are then collected onto a streptavidin-coated microtitration well, and the bound Eu3+ is measured in a time-resolved fluorometer. This assay is rapid, user friendly, and quantitative and lends itself to automation. The application of this assay to the detection of human immunodeficiency virus type 1 is described.

PMID: 1890180, UI: 91365881

Anal Chem 1993 Jan 15;**65(2)**:158-163

Quantification of polymerase chain reaction products in agarose gels with a fluorescent europium chelate as label and time-resolved fluorescence spectroscopy.

Chan A, Diamandis EP, Krajden M

Department of Clinical Biochemistry, Toronto Hospital, Ontario, Canada.

We have 5'-end-labeled one polymerase chain reaction (PCR) primer with the europium chelator 4,7-bis(chlorosulfophenyl)-1,10-phenanthroline-2,9-dicarboxylic acid (BCPDA). After performing PCR in the presence of another unlabeled primer, we separated the 362 bp PCR product with 2% low melting point agarose gel electrophoresis. The gel was then immersed into a Eu3+ solution. During soaking, Eu3+ diffuses into the gel and associates with BCPDA to form a fluorescent complex of long fluorescence lifetime. This complex can be quantified by scanning the gel with a time-resolved fluorometric reader. Because BCPDA and Eu3+ are not fluorescent by themselves, background signals are very low. The detection limit was about 5 ng of DNA. We have also shown that the BCPDA-labeled product could be blotted and detected on the membrane by using an anti-BCPDA antibody. These two technologies may find applications other than in PCR, e.g. in fluorescence-based DNA sequencing and in solution hybridization.

PMID: 7679258, UI: 93158897

Clin Biochem 1994 Oct;**27(5)**:373-379

Detection of Philadelphia chromosome using PCR and europium-labeled DNA probes.

Eskola JU, Hamalainen M, Nanto V, Rajamaki A, Dahlen P, Iitia A, Siitari H

Joint Clinical Biochemistry Laboratory, University of Turku, Turku University Hospital, Finland.

More than 95% of the patients with chronic myelogenous leukemia (CML) carry translocations between protooncogene abl of chromosome 9 and bcr gene of chromosome 22, resulting in the Philadelphia chromosome (Ph1). After allogeneic bone marrow transplantation (BMT) it is important to detect possible residual malignant cells in CML patients. A new sensitive hybridization method combined with polymerase chain reaction (PCR), based on the detection of the europium (Eu3+) label by time-resolved fluorescence, was applied for the detection of Ph1 chromosome. Total RNA from 10(6) peripheral blood leukocytes was isolated by the acid guanidinium thiocyanate-phenol-chloroform extraction. After cDNA synthesis by reverse transcriptase, the PCR amplification (30 cycles) was carried out. In the detection phase two oligonucleotide probes were used in the hybridization reaction, one biotinylated (bcr gene, exon 2) and one (abl gene) labeled with Eu3+. The hybrids were collected in a streptavidin-coated microtitration well and the bound Eu3+ was measured in a time-resolved fluorometer. To assess the sensitivity of the method, different numbers of CML cell line K562 cells were mixed with 10(5) apparently normal human leukocytes. Five K562 cells/10(5) leukocytes could be detected. Six patients with CML confirmed by clinical and cytogenetic criteria were studied. Three of the patients underwent an allogeneic BTM 6-18 months before the investigation and all of them were Ph1-negative. The other three patients who were nontransplanted were positive as expected.

PMID: 7867215, UI: 95171612

J Clin Microbiol 1993 Jul;**31(7)**:1886-1891

Detection of adenovirus in clinical specimens by polymerase chain reaction and liquid-phase hybridization quantitated by time-resolved fluorometry.

Hierholzer JC, Halonen PE, Dahlen PO, Bingham PG, McDonough MM

Respiratory and Enteric Viruses Branch, Centers for Disease Control, Atlanta, Georgia 30333.

In addition to tests for the group-specific hexon antigen of adenoviruses, adenoviruses can be detected in clinical specimens by hybridization assays utilizing the widely shared base sequences of the region of the hexon gene that codes for the group-reactive determinants. We have developed a liquid-phase hybridization system with biotin- and europium-labeled probes which are reacted after DNA amplification of a 161-bp region of the hexon gene and which are quantitated by time-resolved (TR) fluorometry in streptavidin-coated microtiter wells. Polymerase chain reaction (PCR)-TR fluorometry is not a rapid test in the usual sense, but it is highly useful for specimens with inherent toxicity or with low virus yield, such as organ minces and specimens obtained late in the course of an illness. In a survey of 103 specimens tested by this method, including urine, stool, and tissue suspensions, the agreement with the hexon-specific TR fluoroimmunoassay antigen test for positive specimens was 100% and the sensitivity compared with that of virus culture was 91%. The PCR-TR fluorometry system was also shown to be advantageous as a quantitative measure of PCR products.

PMID: 8349768, UI: 93352755

Clin Chem 1993 Feb;**39(2)**:196-201

Europium(III) trisbipyridine cryptate label for time-resolved fluorescence detection of polymerase chain reaction products fixed on a solid support.

Lopez E, Chypre C, Alpha B, Mathis G

CIS Bio International, Laboratoire des Produits pour l'Analyse Medicale, Bagnols sur Ceze, France.

We report a method for the nonradioactive detection of specific amplified target DNA, based on a new long-lived fluorescent label, europium(III) trisbipyridine cryptate [TBP-(Eu3+)]. After a polymerase chain reaction with a set of nested primers, microtiter affinity-collected hybrids were recognized by a TBP(Eu3+)-labeled antibody. The particular structure of the label containing the energy transfer units (bipyridine moieties) enables direct fluorescence measurement on the solid phase. Moreover, its stability and long lifetime contribute to the enhancement of the signal-to-noise ratio. The usefulness of this new label is illustrated by the detection of human papillomavirus type 16 DNA in clinical smears.

PMID: 8381731, UI: 93161526

Nucleic Acids Res 1991 Jan 11;**19(1)**:109-116

Post-PCR sterilization: development and application to an HIV-1 diagnostic assay.

Isaacs ST, Tessman JW, Metchette KC, Hearst JE, Cimino GD

HRI Research, Inc., Berkeley, CA 94710.

We have developed an effective post-PCR sterilization process and have applied the procedure to a diagnostic assay for HIV-1. The method, which is based on isopsoralen photochemistry, satisfies both the inactivation and hybridization requirements of a practical sterilization process. The key feature of the technique is the use of isopsoralen compounds which form covalent photochemical adducts with DNA. These covalent adducts prevent subsequent extension of previously amplified sequences (amplicons) by Taq polymerase. Isopsoralens have minimal inhibitory effect on the PCR, are activated by long wavelength ultraviolet light, provide sufficient numbers of covalent adducts to impart effective sterilization, modify the amplified sequence such that it remains single-stranded, and have little effect on subsequent hybridization. The sterilization procedure can be applied to a closed system and is suitable for use with commonly used detection formats. The photochemical sterilization protocol we have devised is an effective and pragmatic method for eliminating the amplicon carryover problem associated with the PCR. While the work described here is limited to HIV-1, proper use of the technique will relieve the concern associated with carryover for a wide variety of amplicons, especially in the clinical setting.

PMID: 2011490, UI: 91187605

Nucleic Acids Res 1991 Jan 11;**19(1)**:99-107

Post-PCR sterilization: a method to control carryover contamination for the polymerase chain reaction.

Cimino GD, Metchette KC, Tessman JW, Hearst JE, Isaacs ST

HRI Research, Inc., Berkeley, CA 94710.

We describe a photochemical procedure for the sterilization of polynucleotides that are created by the Polymerase Chain Reaction (PCR). The procedure is based upon the blockage of Taq DNA polymerase when it encounters a photochemically modified base in a polynucleotide strand. We have discovered reagents that can be added to a PCR reaction mixture prior to amplification and tolerate the thermal cycles of PCR, are photoactivated after amplification, and damage a PCR strand in a manner that, should the damaged strand be carried over into a new reaction vessel, prevent it from functioning as a template for the PCR. These reagents, which are isopsoralen derivatives that form cyclobutane adducts with pyrimidine bases, are shown to stop Taq polymerase under conditions appropriate for the PCR process. We show that effective sterilization of PCR products requires the use of these reagents at concentrations that are tailored to the length and sequence of the PCR product and the level of amplification of the PCR protocol.

PMID: 2011516, UI: 91187655

J Clin Microbiol 1993 Sep;**31(9)**:2356-2360

Preventing false positives: quantitative evaluation of three protocols for inactivation of polymerase chain reaction amplification products.

Rys PN, Persing DH

Division of Clinical Microbiology, Mayo Clinic, Rochester, Minnesota 55905.

False-positive results because of carryover contamination by previously amplified nucleic acids are currently the greatest impediment to routine implementation of nucleic acid amplification protocols. We evaluated three methods for inactivation of a 156-bp Borrelia burgdorferi polymerase chain reaction (PCR) product: (i) post-PCR cross-linking with isopsoralen (IP), (ii) pre-PCR treatment of a dU-containing PCR product with uracil N-glycosylase (UNG), and (iii) post-PCR alkaline hydrolysis (primer hydrolysis) of PCR products synthesized by using primers containing 3' ribose residues. The sensitivities of the PCR performed under the conditions of each protocol were comparable. Inactivation of amplified DNA was highly efficient for all three protocols; the IP and UNG protocols eliminated at least to 3 x 10(9) copies of the product. The primer hydrolysis protocol varied in efficiency depending on the number and position of the 3' ribose residues, but inactivation ranged from 10(4) to 10(9) copies. We conclude that with some modifications, all three systems are effective for eliminating amplified DNA products. Routine implementation of at least one method should help to avoid false-positive results because of carryover contamination.

PMID: 8408555, UI: 94013388

J Clin Microbiol 1996 Dec;**34(12)**:3115-3119

Use of AmpliWax to optimize amplicon sterilization by isopsoralen.

De la Viuda M, Fille M, Ruiz J, Aslanzadeh J

Department of Laboratory Medicine, University of Connecticut School of Medicine, Farmington 06030, USA.

The photochemical inactivation of amplicons by isopsoralen (IP-10) has been suggested as a possible means to prevent PCR carryover contamination. To evaluate the technique, serial dilutions of amplicons (10(11) to 10(3)) from the Borrelia burgdorferi OSP A gene were amplified in the presence of 0, 25, 50, and 100 micrograms of IP-10 per ml for 45 cycles. The PCR products were exposed to UV light for 15 min to activate IP-10 and sterilize the amplicons. One microliter of each sterilized sample was reamplified for an additional 45 cycles. The PCR products were then resolved in an agarose gel, blotted onto a nylon membrane, and probed with an alkaline phosphatase-conjugated chemiluminescent probe. Although IP-10 at concentrations of 50 and 100 micrograms/ml effectively sterilized up to 10(11) amplicons, the compound was inhibitory to PCR. IP-10 at a concentration of 25 micrograms/ml had slight inhibitory effect on PCR and did not completely sterilized all of the amplicons. Therefore, in subsequent experiments AmpliWax was substituted for mineral oil, and PCR was performed on 10(9) to 10(3) amplicons as described above. Following the amplification, the PCR tubes were cooled to solidify the AmpliWax and inoculated with various concentrations of IP-10. With this technique, PCR products produced from as many as 10(9) target amplicons were effectively sterilized with 200 micrograms of IP-10 per ml. Similarly, the addition of IP-10 (50 micrograms/ml) before and after PCR was evaluated for the detection of B. burgdorferi in 62 ticks from a region of Southern Connecticut where the organism is highly endemic. PCR performed in the presence of 50 micrograms of IP-10 per ml detected B. burgdorferi-specific DNA in 17 of 62 ticks (27%) following gel electrophoresis and in 34 of 62 ticks (55%) following Southern blot hybridization of the PCR products. In contrast, post-PCR addition of IP-10 detected borrelia-specific DNA in 31 of 62 ticks (50%) following gel electrophoresis and in 46 of 62 ticks (64%) following Southern blot hybridization. We conclude that the replacement of mineral oil with AmpliWax can be useful in eliminating the inhibitory effects of IP-10 and other sterilizing agents for post-PCR sterilization of amplicons.

PMID: 8940457, UI: 97095036

1: *J Protozool* 1989 Jan-Feb;36(1):60S-61S Related Articles, Books

Analysis of a surface antigen of Pneumocystis carinii.

Linke MJ, Walzer PD

VA Medical Center, Cincinnati, OH.

The 115 kd band in polyacrylamide gels is a major antigen of Pneumocystis carinii. Data obtained from treatment with enzymes, binding to lectins, and labelling the surface with biotin suggest that this moiety is a glycoprotein containing mannosyl/glucosyl and N-acetylglucosamine residues, and that it is located on the cell wall of the organism. Other rat and human P. carinii antigens also are glycoproteins but differ in specific protein or carbohydrate residues or location on the organism.

PMID: 2785200